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Adenovirus Hexon Monoclonal Antibody That Is Group Specific and Potentially Useful as a Diagnostic Reagent

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A monoclonal antibody to the adenovirus 2 hexon protein was produced and characterized as a group-specific antibody. Positive reactivity in immunoprecipitation, indirect immunofluorescence, and radioimmunoassays was observed with human, canine, swine, bovine, murine, and simian adenoviruses. This monoclonal antibody should provide a specific and sensitive diagnostic reagent for detection of all mammalian adenoviruses.

All mammalian adenoviruses share antigenic domains, which are known as group-specific determinants (13, 14). At least one of the group-specific determinants is located on the major capsid protein, hexon. We have produced three monoclonal antibodies to hexon, designated 2Hx-1, 2Hx-2, and 2Hx-3, as well as monoclonal antibodies to several other structural and nonstructural adenovirus proteins (3). The data presented here (and in reference 3) indicate that the antihexon monoclonal antibodies are group specific and are suitable for use as highly specific, high-affinity reagents for detection of mammalian adenoviruses with several diagnostic techniques.

The capsomeric form of hexon is a trimer of 12 S which consists of three identical monomers of 100,000 molecular weight each (9). The native trimer does not share any antigenic determinants with the individual monomers or denatured hexon (19). Similarly, group-specific antisera do not recognize denatured hexon (5). Neutralization of virus infectivity and reactivity with intact virion particles cannot be demonstrated with group-specific sera (5). However, gentle disruption of virions releases antigens that are reactive with group-specific sera. We used immunoprecipitation techniques to investigate the reactivity of our antihexon monoclonal antibodies with all of these antigenic forms. Previously, we demonstrated that 2Hx-1 exhibited all of the biochemical specificities of group-specific antisera (3). As shown here (Fig. 1), 2Hx-2 also exhibited specificity for native, 12S, hexon capsomeres. We also demonstrated that monoclonal 2Hx-3 had the same specificity as 2Hx-1 and 2Hx-2 (data not shown). All three antibodies failed to immunoprecipitate [^{35}S]methionine-labeled purified adenovirus 2 (Ad2) virions and to neutralize virus infectivity; however, all were reactive with disrupted virions (Fig. 1, lane M; reference 3). Two of these antihexon hybrids were independently derived from mice immunized with either purified Ad2 virions (2Hx-1) or Ad2-infected HeLa cell extracts (2Hx-2 and 2Hx-3). Clone 2Hx-2 produced ascites fluid with the highest titer and was thus chosen for further characterization.

Human, simian, canine, porcine, murine, bovine, avian, and amphibian adenoviruses have been identified (15, 20). All of the adenoviruses have been shown to share antigenic determinants, with the exception of avian and amphibian adenoviruses (4, 13, 14). The reactivity of 2Hx-2 with many different human and animal isolates was investigated by immunoprecipitation, indirect immunofluorescence (IF), and radioimmunoassay (RIA) techniques. Immunoprecipitation of adenovirus-infected human and monkey cell extracts is shown in Fig. 2. Monoclonal 2Hx-2 was reactive specifically with the hexon protein of a representative serotype of each human subgroup as well as the simian adenovirus, SA7. The observed molecular weights of the immunoprecipitated hexons were in each case consistent with the reported molecular weights (20). The affinity of 2Hx-2 for the hexon of the different serotypes was comparable to that observed for Ad2 or Ad5. The variation in the amount of hexon immunoprecipitated in each case was a reflection of the amount of hexon in each extract, which was estimated by electrophoresis of the total cell extract on a parallel gel (data not shown).

When 2Hx-2 was used as the primary antibody for IF on cells infected with a variety of human and animal adenoviruses, nuclear fluorescence was observed (Fig. 3). Positive results

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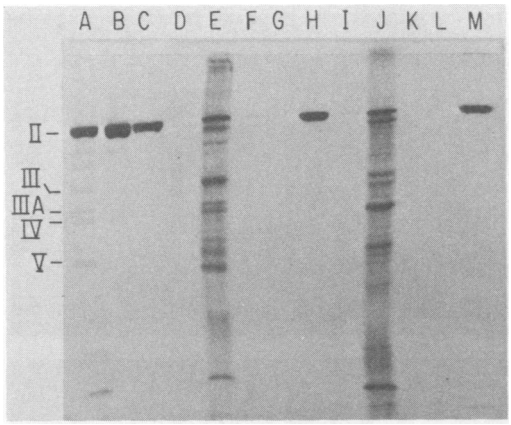

FIG. 1. Immunoprecipitation analysis of 2Hx-2 specificity. The specificity of 2Hx-2 for hexon conformation was determined by immunoprecipitation of various antigen preparations. The antigens were prepared as described in reference 3 and immunoprecipitated with *Staphylococcus aureus* (11). The input and immunoprecipitated antigens were electrophoresed on a 7.5% sodium dodecyl sulfate-acrylamide gel (12) which was fluorographed with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) and exposed on preflashed Kodak XAR-5 film (2). (A) Purified [^{35}S]-methionine-labeled Ad5 virion markers (structural proteins II [hexon], III, [penton base], IIIA, and IV [fiber] and core protein V). (B) 1 μl of purified hexon trimers. (The trimers were shown to be 12S by centrifugation on sucrose gradients.) (C and D) Immunoprecipitation of hexon trimers (2 μl) shown in (B) by 2Hx-2 (C) and nonimmune mouse serum (D). (E) Total cell extract (15 μl) of Ad5-infected cells pulsed with [35S]-methionine 18 to 19 h postinfection. (F and G) Total cell extract (as in [E]) was denatured by boiling in 2.0% sodium dodecyl sulfate and 0.5% mercaptoethanol and immunoprecipitated by 2Hx-2 (F) and nonimmune mouse serum (G). (H and I) Immunoprecipitation of total cell extract (E) by 2Hx-2 (H) and nonimmune mouse serum (I). (J) In vitro translation products (2 μl) of total cytoplasmic RNA from Ad5-infected HeLa cells. (The hexon produced by in vitro translation was shown to be monomeric by centrifugation in sucrose gradients.) (K and L) Immunoprecipitation of in vitro translation products (5 μl) by 2Hx-2 (K) and nonimmune mouse serum (L). (M) Immunoprecipitation product of virions disrupted by sodium dodecyl sulfate (17) and by 2Hx-2.

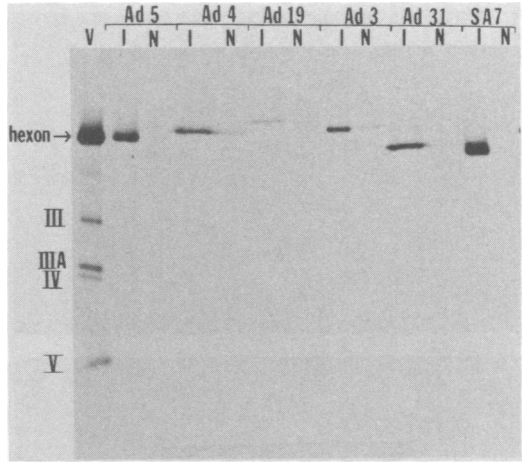

FIG. 2. Immunoprecipitation of hexon of different human and simian adenoviruses. The 293 line of cells of Graham et al. (7) were infected with the indicated serotypes and pulse-labeled with [^{35}S]methionine at 16 to 17 h postinfection. Total cell extracts were prepared and immunoprecipitated with *S. aureus* (3, 11). Immunoprecipitations with 2Hx-2 (I) and nonimmune serum (N) are shown. Lane V shows purified [^{35}S]methionine-labeled Ad5 virion markers. Other lanes are the result of immunoprecipitation of different human subgroups, as indicated. SA7, Simian adenovirus 7, which was grown on CV-1 cells. See legend to Fig. 1 for explanation of III, IIIA, IV, and V.

were obtained with isolates that infect humans (subgroups A through E), monkeys (SA7), dogs (CAV-1, CAV-2), cattle (BAV-1), and swine (SAV). The only negative results encountered to date were with the avian adenovirus, CELO, which has been reported to lack the group reactivities (4). A summary of these data is given in Table 1. The reactivity of 2Hx-2 with these animal and human serotypes was also investigated with RIA. The IF results were confirmed with RIA (Table 1).

The vertex capsomere, penton base, has also been reported to exhibit group-specific determinants (14). Investigations of our anti-penton base monoclonal antibodies, 2Pb-1, 2Pb-2, and 2Pb-3, indicated that these reagents were specific for the human subgroup C, as was our anti-100,000-molecular-weight protein monoclonal antibody, 2100K-1. Antifiber monoclonal antibodies, 2Fb-1, 2Fb-2, and 2Fb-3, were found to be serotype specific for Ad2.

How many determinants were conserved in a group-specific manner on the hexon capsomere and where are these determinants located? All three of our antihexon monoclonal antibodies, and mixtures thereof, were found to be negative in double-diffusion assays (Ouchterlony) when tested against purified trimers or extracts from infected cells. This suggests that all three antibodies bind to the same general site. A less likely possibility, given the extraordinary stability of the hexon trimer, is that binding of one antibody molecule perturbs the hexon structure and prevents the binding of a second antibody molecule. The group-reactive site is not located on the outer surface of the virion particle. All three of the antihexon monoclonal antibodies, and group-specific antisera, did not react with intact virions, but did react with disrupted virions. The hexon trimer thus either undergoes a

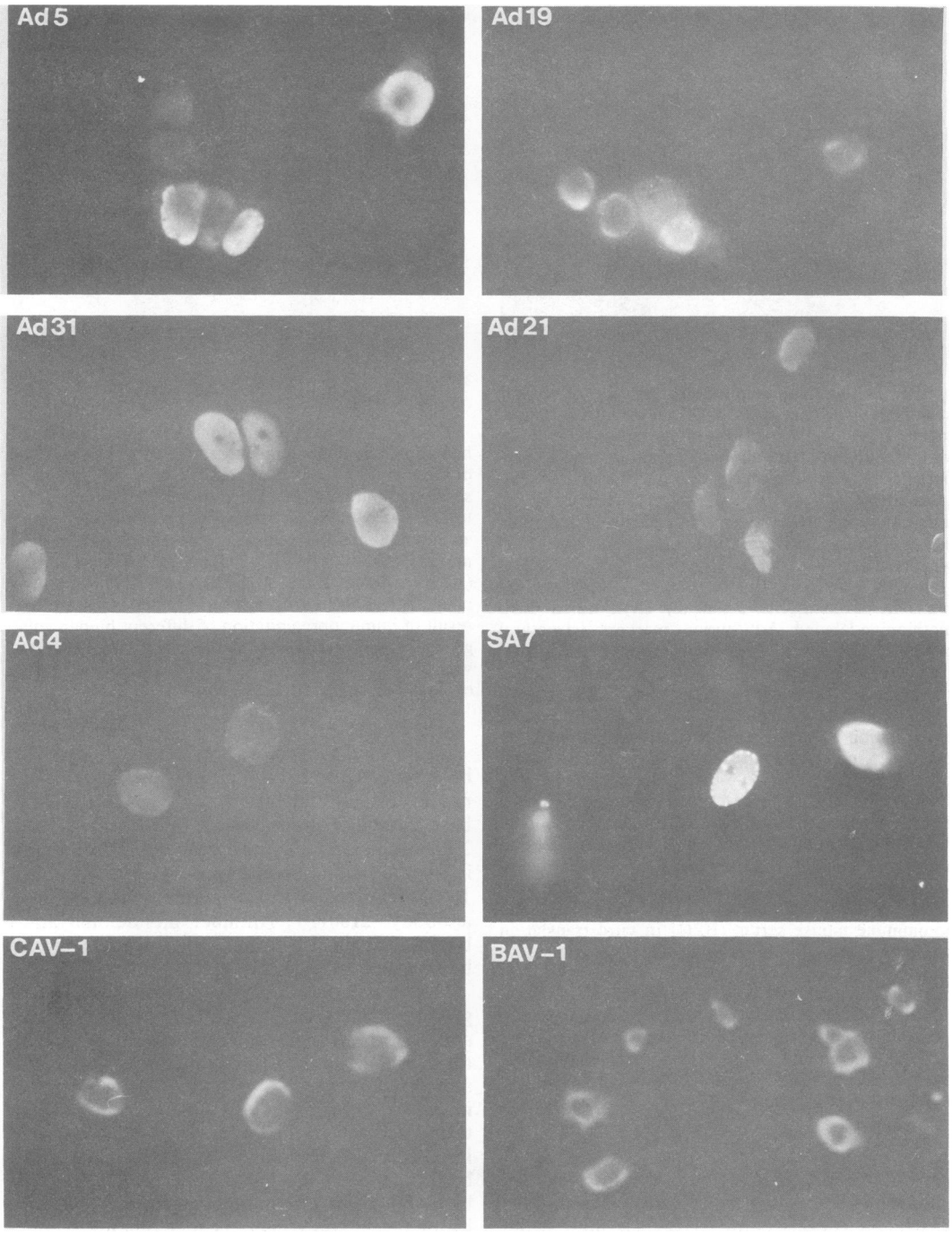

FIG. 3. IF of human and animal adenoviruses. Cells were plated on glass cover slips, infected, fixed at 29 h postinfection with 3.7% formaldehyde in phosphate-buffered saline, and permeabilized with acetone as described previously (18). Cells were incubated with 2Hx-2 ascites fluid (diluted to 10^{-3}) as primary antibody for 30 min at 37°C and with fluorescein-conjugated rabbit anti-mouse IgG (Miles Laboratories, Inc., Elkhart, Ind.) as secondary antibody. The UV photomicrographs are shown at a magnification of ×850. IF was negative when infected cells were stained with normal mouse serum. The serotypes are as indicated on the photographs, and the host cells are as listed in Table 1. For a summary of IF results, see Table 1.

TABLE 1. Specificity of 2Hx-2 in RIA and by IF[a]

Serotype (subgroup)	Host species (cell line)	RIA[b]	IF[b]	Serotype (subgroup)	Host species (cell line)	RIA[b]	IF[b]
Ad2 (C)	Human (HeLa)	+	+	SA7	Simian (CV-1)[d]	+	+
Ad5 (C)	Human (HeLa)	+	+				
Ad1 (C)	Human (293)[c]	+	+	CAV-1	Canine (1° DK)[e]	+	+
Ad31 (A)	Human (293)	+	+	CAV-2	Canine (1° DK)	+	+
Ad21 (B)	Human (293)	+	+				
Ad11 (B)	Human (293)	+	+	BAV-1	Bovine (1° EBK)[f]	+	+
Ad3 (B)	Human (293)	+	+				
Ad7 (B)	Human (293)	+	+	SAV	Swine (1° SKP)[g]	+	+
Ad19 (D)	Human (293)	+	+				
Ad10 (D)	Human (293)	+	+	MAV	Murine (1° ME)[h]	+	ND[i]
Ad4 (E)	Human (293)	+	+				
				CELO	Avian (1° CEF)[j]	−	−

[a] The animal adenoviruses CAV-1, CAV-2, BAV, SAV, and CELO were from the National Veterinary Services Laboratories in Ames, Iowa. The human adenoviruses, except Ad2, Ad5, Ad3, and Ad7, were provided by Larry Anderson and Kenneth McIntosh of the Children's Hospital Medical Center of Boston, Mass. The murine adenovirus was from Hilton Klein of Microbiological Associates Bioproducts of Walkersville, Md. Virus stocks were prepared as described previously (3). These stocks were diluted 1:10 in 0.15 M NaCl–10 mM sodium phosphate, pH 7.4, heated for 10 min at 56°C to gently lyse virions, centrifuged for 5 min at 15,000 rpm, and used as antigens for RIA as described previously (3). Primary antibodies were 2Hx-2 ascites fluid (10^{-3} to 10^{-4} dilution), nonimmune mouse ascites fluid, goat anti-Ad5 group-specific serum from Microbiological Associates Bioproducts, or nonimmune goat serum. Secondary antibodies were prepared by purifying rabbit anti-mouse IgG and rabbit anti-goat IgG on DEAE Affi-Gel Blue columns (Bio-Rad Laboratories, Richmond, Calif.) and iodinating by using the chloramine-T method (8). Specific activities were routinely 1×10^7 to 2×10^7 cpm of ^{125}I per μg of protein. An assay with 2Hx-2 was considered positive if there were at least as many counts per minute bound on infected cell extracts as were bound by goat anti-Ad5 serum. The specific binding of 2Hx-2 to positives varied between 4 to 100 times that of background levels. Positive IF gave distinctive nuclear staining of infected cells as compared with uninfected cells (3). Uninfected cells stained with 2Hx-2 and infected cells stained with nonimmune mouse serum gave no detectable IF.

[b] +, Positive reaction; −, no reaction.

[c] Cell line 293, transformed human embryonic cell line of Graham et al. (7).

[d] Monkey kidney cell line.

[e] Primary dog kidney cells.

[f] Primary embryonic bovine kidney cells.

[g] Primary swine kidney cells.

[h] Primary Swiss mouse embryo cells.

[i] ND, Not determined.

[j] Primary chicken embryo fibroblasts.

reversible conformational change when it becomes part of the capsid structure, or the group-specific site is localized on the interior of the capsid where conserved interactions presumably take place.

The hexon group determinant is probably formed by the folding together of distal parts of either one or more hexon polypeptide chains, as these monoclonal antibodies show no cross-reactivity with either denatured hexon or native hexon monomers prepared by in vitro translation. The only hexon amino acid sequence that has been determined is that of Ad2 (10). Given that the group determinant may not be formed by contiguous sequences, determination of the chemical structure of the hexon capsomere by X-ray diffraction may be necessary to identify the binding site of these monoclonal antibodies. The structure of the hexon trimer of 0.6 nm resolution has been reported, and the interpretation of a 0.29 nm map is currently under way (1).

Few viral mutations that change the structure of this highly conserved determinant are viable. A chemical compound with a high affinity for the group-specific site would probably prevent virion assembly and block further infection. This would be equivalent to the binding of colchicine to the highly conserved tubulin monomer (16). A drug which can bind to the group-specific determinant should be expected to have a broad spectrum of antiadenovirus activity. In addition, few viral mutants resistant to its activity would be expected. Perhaps it would be possible to use a group-specific monoclonal antibody to identify such a drug.

The fact that 2Hx-2 immunoprecipitates most of the input hexon antigen, and only the hexon antigen, from a variety of serotypes indicates that 2Hx-2 is a high-affinity reagent, as well as a highly specific one. The distinctive nuclear fluorescence that is characteristic of adenovirus-infected cells stained with 2Hx-2 may allow

diagnosis to be carried out directly on cells taken from the patient. Alternatively, RIA or enzyme-linked immunosorbent assay may be performed on clinical specimens. Monoclonal antibodies to some viruses are limited in their usefulness as broadly reactive diagnostic reagents since monoclonal antibodies by their very nature recognize a limited domain of a protein. However, since adenoviruses have evidently retained the 2Hx-2 determinant during their evolution as mammalian viruses, the availability of a monoclonal reagent that recognizes such a highly conserved determinant should aid in positive identification of isolates as adenoviruses.

Clone 2Hx-2 is available from the American Type Culture Collection, Rockville, Md. as culture number HB 8117.

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